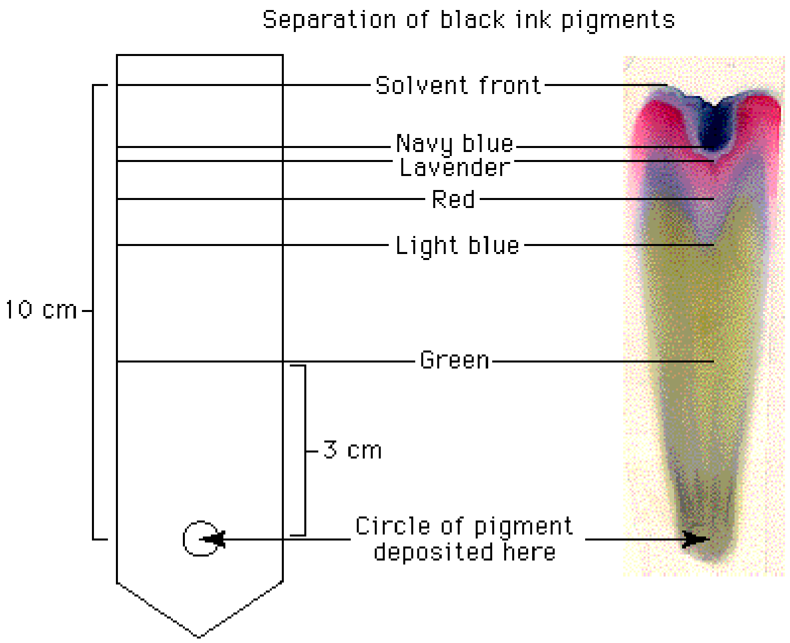
Key Concepts I: Plant Pigment Chromatography

Paper chromatography is a technique used to separate a mixture into its component molecules. The molecules migrate, or move up the paper, at different rates because of differences in solubility, molecular mass, and hydrogen bonding with the paper.

For a simple, beautiful example of this technique, draw a large circle in the center of a piece of filter paper with a black water-soluble, felt-tip pen. Fold the paper into a cone and place the tip in a container of water. In just a few minutes you will have tie-dyed filter paper!



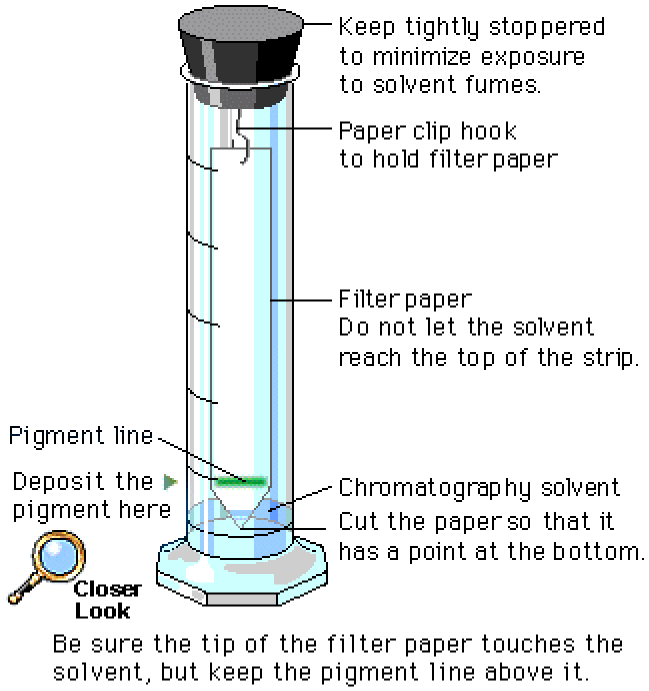
The green, blue, red, and lavender colors that came from the black ink should help you to understand that what appears to be a single color may in fact be a material composed of many different pigments — and such is the case with chloroplasts. **chloroplast**

(**klor**-oh-plast) [Gk. *chloros,* green + *plastos,* formed]
An organelle found only in plants and photosynthetic protists that absorbs sunlight and uses it to drive the synthesis of organic compounds from carbon dioxide and water.

Design of the Experiment I

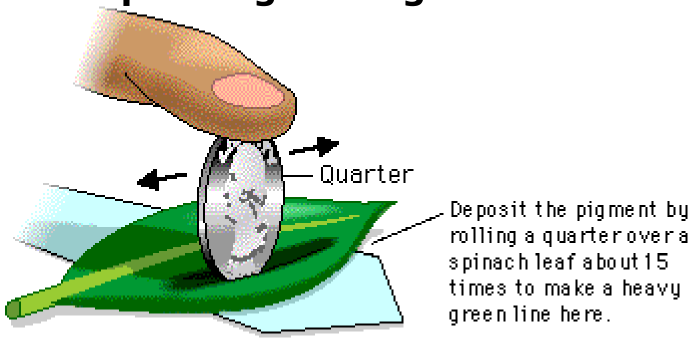
In paper chromatography the pigments are dissolved in a solvent that carries them up the paper. In the ink example, the solvent is water. To separate the pigments of the chloroplasts, you must use an organic solvent.

 In the following activity, you will separate plant pigments using an organic solvent such as a mixture of ether and acetone. Be sure to keep the bottle tightly closed except when you are using it because the solvent is very volatile and produces fumes you should not breathe.



The next screen shows you the separation of plant pigments.

Depositing the Pigment



Analysis of Results I

If you did a number of chromatographic separations, each for a different length of time, the pigments would migrate a different distance on each run. However, the migration of each pigment relative to the migration of the solvent would not change. This migration of pigment relative to migration of solvent is expressed as a constant, R_f (Reference front). It can be calculated by using the formula:

$$R_f = \frac{\text{distance pigment migrated}}{\text{distance solvent front migrated}}$$

Look back at the black ink chromatogram, and then calculate the R_f value for green

Name………………………………………………………………………….. Date………………….. Class………..

Oxygen and Photosynthesis

Green plants can turn chemicals into food. Green plants take in water and carbon dioxide and, in the presence of light and chlorophyll, turn these chemicals into food. This process is called photosynthesis. One of the by-products of photosynthesis is oxygen. The amount of oxygen produced by a plant during a period of time can serve as a way of telling how much photosynthesis is taking place.

Strategy

You will place a plant under continuous light conditions for 24 h while another plant remains in the dark for the same amount of time.

You will compare the amount of photosynthesis that takes place in these two plants by measuring the amount of oxygen the plants produce.

Materials

WARNING: *Do not taste, eat, or drink any materials used in the lab.*

2 glass jars (large enough to hold the funnels)
aged tap water (standing for at least 24 h)
sodium bicarbonate (baking soda)
balance
Elodea
metric ruler
scissors
2 glass funnels (small)
2 test tubes (18 × 150-mm)
lamp

Procedure

1. Fill each jar with water that has been standing for at least one day. Add 1 g of sodium bicarbonate to the water in each jar.

2. Obtain two *Elodea* plants and cut about 1 or 2 cm from the bottom of the stem. Throw away the part you cut off.

WARNING: *Always be careful when using scissors.* Lightly crush the upper 2.5 cm of the stem between your fingers.

3. Place an *Elodea* plant into the water in each jar and cover it with a funnel. Position the plants so that the crushed ends are up. (see figure 1)

4. Fill a test tube completely with water. Hold your index finger over the mouth of the test tube and invert it over the stem of the funnel. Do not let any water escape from the test tube. **NOTE:** The test tube must be completely filled with water at the beginning of the experiment. If some water pours out before the test tube is in place, start over again. Do not remove your finger until the mouth of the test tube is completely under water. Place a test tube over each funnel. (see figure 1)

5. Place one jar near a bright light where it will remain in light for 24 h. Place the other jar in the dark. The one in the dark is the control.

6. After 24 h, measure the height in centimeters of the gas column that collected in each test tube.

Figure 1

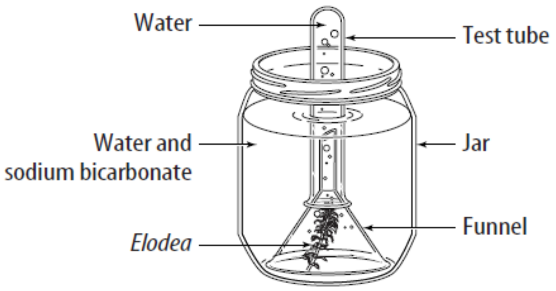
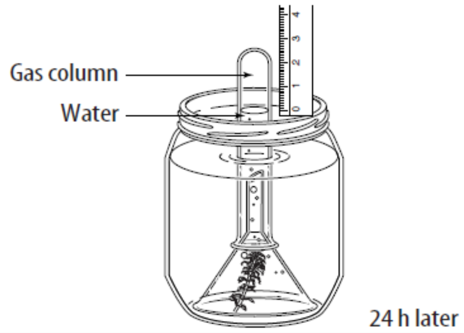


Figure 2



7. In Table 1, record the height of the gas column you measured in the test tube for each plant.
8. Compare the data table with the average gas column height measured by your class.

Data and Observations

Table 1

	Height of gas column	
Plant	**My results**	**Class average**
1. In light		
2. In dark		

Questions and Conclusions

1. What proof do you have that light is needed for photosynthesis?

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____

2. What proof do you have that oxygen is being given off during this experiment? Before you answer, carefully review what you observed during this experiment.

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3. Why was sodium bicarbonate added to the water? HINT: Sodium bicarbonate gives off carbon dioxide when mixed with water.

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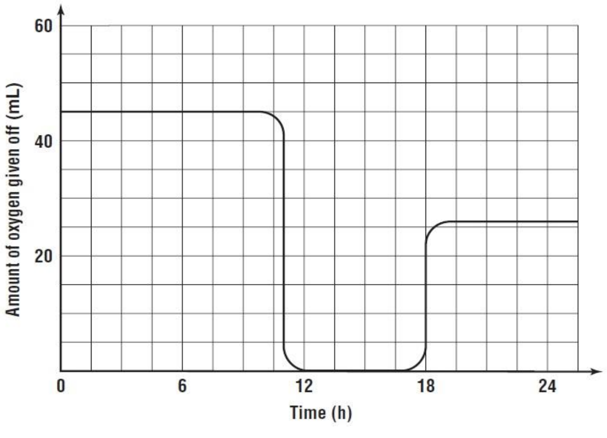
The graph below shows the amount of oxygen given off by a plant during a 24-h time period



4. a. How many hours did the plant receive light?

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b. How many hours was the plant in the dark?

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c. How many milliliters of oxygen were given off between hours 18 and 24?

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5. Explain what change may have taken place in the light during hours 18–24 that would have decreased the amount of oxygen given off when compared with hours 0–11.

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6. Which graph below best shows the total amount of oxygen produced if light were shined on a plant for 24 continuous hours?

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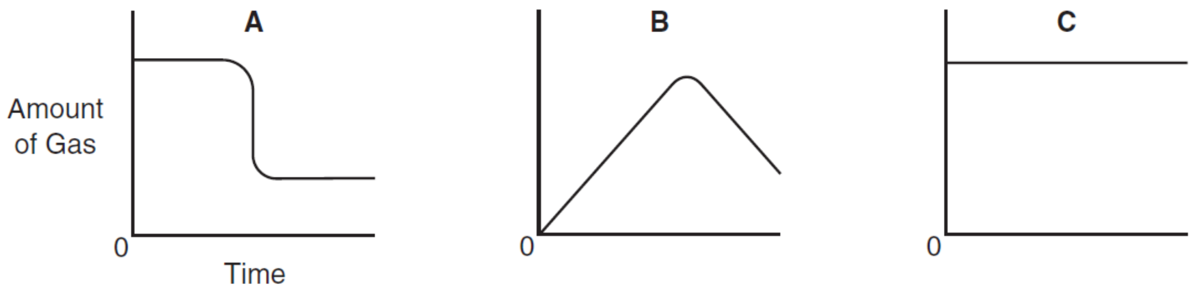
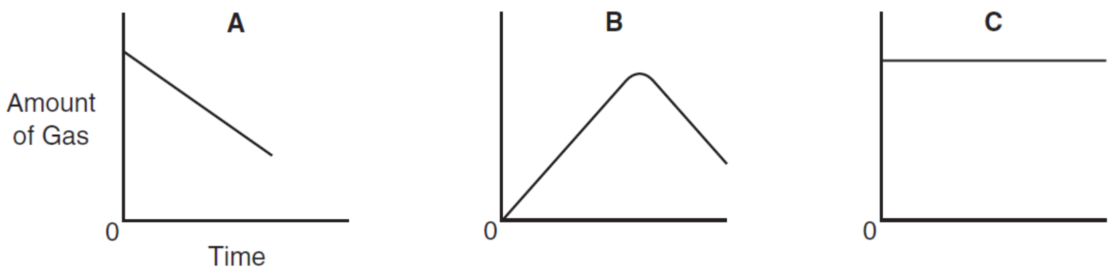


7. Which graph below best shows the total amount of oxygen produced if a light source were slowly moved farther and farther away from the plant during a 24-h period?____________________________



8. From this activity, what are the requirements for photosynthesis to occur?

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9. Write the equation for photosynthesis.

____

10. What are the products of photosynthesis?

____

11. What would happen if there were no green plants?

____

____

12. How does the equation for photosynthesis compare with the equation for respiration?

____
____
____
____

13. Where is chlorophyll found in plants?

____

14. In respiration, what food is most easily broken down by cells?

____

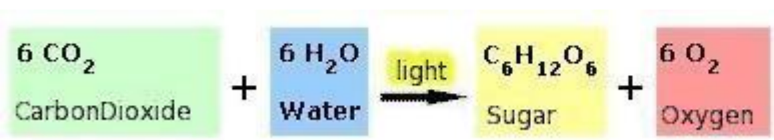
Strategy Check

Can you measure the amount of oxygen that a plant in light and a plant in dark produce?
Can you compare the amount of photosynthesis that takes place in each plant?

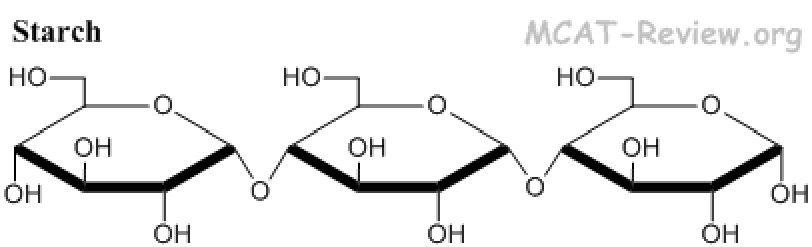
Sugar Makers

Plants contain the same biological processes and biochemistry as microbes and animals. However, plants are unique in that they have the ability to use energy from sunlight along with other chemical elements for growth. This process of photosynthesis provides the world's supply of food and energy.

Plants have the amazing ability to convert solar energy into the chemical energy of sugar. They do this through a set of chemical reactions called photosynthesis. The energy of photosynthesis drives our planet. Plants are the primary producers of food within ecosystems and the foundation of the food chain. Therefore, when you eat, you're using the energy from photosynthesis to run your body. Most of our machines also run on "plant power." For example, when you ride in a gas-powered car, you're using energy from photosynthesis which took place millions of years ago (plants were buried and cooked in the Earth to form oil). Photosynthesis occurs in chloroplasts, small organelles in plant cells that contain the light-absorbing chemical chlorophyll. Photosynthesis requires light, water, and also carbon dioxide (CO2), which provides the carbon atoms that make up the product – glucose.



Plants link glucose molecules together as starch for long-term storage. Starch stores a lot of energy and is a very important source of energy for humans and other animals. Seeds, grains and potatoes contain a lot of starch, which is a carbohydrate. Athletes often "carbo-load" before a race by eating lots of starchy foods, whereas people who want to lose weight sometimes go on "low-carb" diets. In this activity you will investigate the photosynthetic activity of leaves by examining them for starch accumulation. Starch is colorless, but it is stained dark blue by the stain potassium iodine.



Activity: Investigating Photosynthesis

Setting up the experiment:

1. Your instructor will provide you with a plant that has been stored in the dark for 24 to 48 hours. During this time, the plant used up its starch reserves (why?).

2. Cover one half of a leaf with dark paper or foil – cover both the front and back of the leaf, using paper clips to attach the cover to the leaf. Illuminate the leaf with a bright light or the sun for several hours to up to four days.

Decolorizing and staining the leaves:

To examine the starch accumulation in your experimentally treated leaves, you first need to remove the green chlorophyll from them.

3. Carefully remove the treated leaf from the plant and place it into a beaker of boiling water for one minute. This step disrupts the cell membranes and makes the staining steps easier.

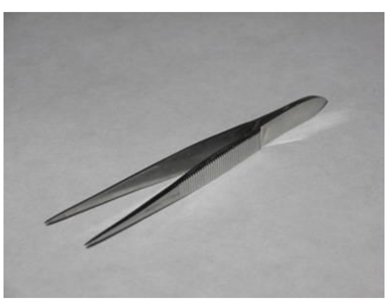
4. Use **tweezers** or **tongs** to transfer the leaf to a beaker containing hot ethanol. Leave it in hot ethanol for three or more minutes. CAUTION – do not use ethanol near a flame!

5. Carefully transfer the leaf to a bowl of cold water – the leaf will be very fragile at this point, so be gentle with it.

6. Drop iodine solution onto the leaf – make sure to cover the whole leaf. Record your observations – do you see any difference in starch accumulation in the light and dark-treated portions of the leaf? What do your results tell you? Is light needed for photosynthesis to occur?

Optional activity – starch in foods:

This can be done before or during the starch staining of leaves. Cover a table with newspaper and then a piece of plastic wrap, followed by white paper as a background. Select a few food items that you think will contain a lot of starch, and some that you do not expect to have starch. Place small pieces of these foods onto the paper, and then place a few drops of the iodine solution onto them. Record your observations – which foods reacted most strongly with the iodine by turning black or bluish-purple? Are these the foods you expected to be rich in carbohydrates?


tweezers


tong

Student-Designed Experiments Using the methods you learned in the activity above and the "Guide for Student Experimentation" below, design and carry out your own inquiry. Question topics you might consider include the role of light, chlorophyll, or carbon dioxide in the formation of starch.

Light requirements for photosynthesis - You can use colored plastic films rather than dark paper or foil to block only some light colors from reaching the leaf. Do you think all colors of light support photosynthesis equally? Why or why not?

Chlorophyll requirement for photosynthesis - You can examine starch production in variegated leaves – leaves that have both green and white patches (i.e. spider plant, coleus and geranium). The white patches in the leaves do not have chlorophyll. How do you expect starch accumulation to compare in white versus green parts of the leaf?

CO2 requirement for photosynthesis – Room air is about 0.03% CO2 (by contrast, air is about 21% O2!). You can artificially raise or lower this amount to determine how the CO2 concentration affects photosynthesis. You will need to place your plant or leaf in a sealed chamber while you expose it to light – you can put a small plant under a large beaker, or use a detached leaf instead. Increase the concentration of CO2 by placing a leaf or a plant in a closed environment containing dry ice (solid CO2), carbonated liquid (soda) or a solution of 5% sodium bicarbonate (baking soda). Or, you can fill balloons with your own exhaled breath (about 4% CO2), and release this enriched air into the plant's environment. To decrease the amount of CO2 in the plant's environment, you can place a beaker containing sodium hydroxide pellets into your sealed environment (caution – do not touch sodium hydroxide pellets with bare skin!). Be sure to do proper controls for these experiments!

Guide for Student Experimentation

Guidelines for Achieving Great Experiments

1. Ask a very specific, testable question.

2. Test a control for comparison (a group that does not receive the experimental treatment).

3. Use a sample size large enough to allow firm conclusions.

4. To understand a whole population, obtain a random sample of that population to avoid bias.

5. Replicate each part of the experiment (at least 3 times).

6. Hold all variables constant between trials except the variable being tested.

7. Collect quantitative data whenever possible.

8. Measure using metric units.

9. Gather data carefully and accurately.

10. Be objective and honest.

Introduction

Question:

Hypothesis:

Materials and Methods

Independent variable:

Dependent variable:

Experimental constants:

Control:

Protocol:

Results

Data collected:

Other observations:

Graph(s):

Discussion

Interpretation of data:

6 **Graph(s):**

Discussion

Interpretation of data:

Conclusions: